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Highly Sensitive and Selective Method for Estimation of Formoterol at Sub-pg/mL in Human Plasma Using Shimadzu LCMS-8060NX

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1. Introduction

Formoterol is an inhaled long-acting beta2-adrenergic receptor agonist used as a bronchodilator in the management of asthma and COPD (refer fig.1 for structure of formoterol). A major clinical advantage of formoterol over other inhaled beta-agonists is its rapid onset of action (2-3 minutes), which is at least as fast as salbutamol, combined with a long duration of action (12 hours)

Following single/multiple dose administration of formoterol, the drug exhibits very low bioavailability and requires a highly sensitive and selective quantification of formoterol from plasma.

In this work, we present a highly sensitive and selective method for the accurate quantification of formoterol in human plasma using Shimadzu LCMS-8060NX triple quadrupole mass spectrometer coupled with Nexera X2 UHPLC.

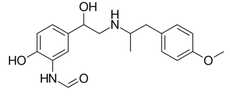

Fig. 1 Structure of Formoterol [(1)]

2. Materials and methods

2-1. Sample Preparation

Calibration standards and quality control samples were prepared in K2 ETDA human plasma with sample concentrations ranging between 0.20-100.00 pg/mL and 0.20-50.00 pg/mL respectively.

Two hundred microliters of extraction buffer was added to plasma samples to enhance the binding of the analyte while performing the solid phase extraction. SPE cartridges were conditioned with 1 mL methanol and equilibrated with 1 mL water. Plasma samples were added to the cartridges and allowed to pass under gravity. 1 mL of 0.05 % ammonia in water and water effectively removed the interferences from SPE cartridges. Formoterol was eluted using 0.2 mL of 10 % acetonitrile in water and was loaded on prelabelled HPLC vials before analysis on LC-MS/MS system

3. LC-MS/MS analysis

LCMS-8060NX coupled with Nexera™ X2 UHPLC system (Shimadzu Corporation), was used to acquire the data in MRM mode. The instrumental conditions used during the analysis are presented below in Table 1

Table 1 Instrument Parameters for analysis of Formoterol

UHPLC condition (Nexera™ X2)	
Column	Shim-packTM Velox C18 100 × 2.1 mm, 2.7 µm (P/N: 227-32015-03)
Mobile phase	A: 0.1% formic acid in 5 mM Ammonium Acetate , B: Acetonitrile
Flow rate	0.30 mL/min
Elution mode	Isocratic
Column temp	50 ℃
MS parameters (LCMS-8060NX)	
MS interface	Electro Spray Ionization (ESI)
Nitrogen gas flow	Nebulizing gas- 3 L/min; Drying gas- 10 L/min
Zero air flow	Heating gas- 10 L/min
MS temp	Desolvation line-200 °C; Heating block- 100 °C;
	Interface- 300 °C

4. Results

4-1. Selectivity

The selectivity of the method was evaluated by extracting and analyzing 6 different lots of blank human plasma. Results are presented in Table 2. Representative chromatogram is shown in fig.2.

Table 2 Selectivity

	Formoterol		
Lot no.	Area in blank matrix	LLOQ area	% Interference
V1102	0	19,359	0.00
V8245	0	17,936	0.00
V6132	0	17,594	0.00
V11886	0	18,480	0.00
V11782	0	23,070	0.00
V11911	0	19,115	0.00

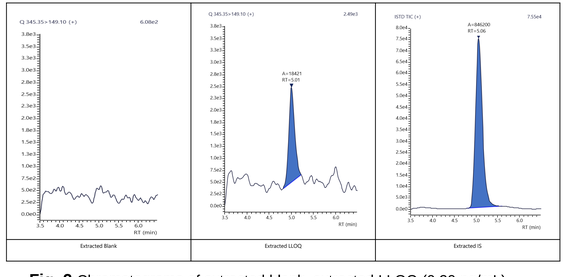

Fig. 2 Chromatograms of extracted blank, extracted LLOQ (0.20 pg/mL) and extracted IS

4-2. Linearity

A eight-point calibration curve with a 1/x^2 weighting factor displayed linearity within the concentration range of 0.20–100.00 pg/mL for formoterol (refer fig.3) resulted in mean correlation coefficient > 0.99.

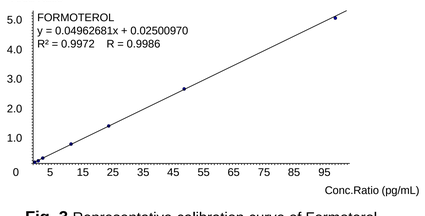

Fig. 3 Representative calibration curve of Formoterol

4-3. Intra-day and Inter-day Precision and accuracy

Intra-day and inter-day precision and accuracy results in plasma quality control samples are summarized in table 3 and table 4 and were found within the acceptance criteria

Table 3 Intra-day Precision and Accuracy

QC level (n=6)	Mean Conc.	% Accuracy	% CV
LLOQ QC (0.20 pg/mL)	0.23	113.60	4.80
LQC (1.57 pg/mL)	1.71	108.82	4.70
MQC (10.00 pg/mL)	9.59	95.89	4.20
HQC (50.00 pg/mL)	49.95	99.90	2.10

Table 4 Global Precision and Accuracy

QC level (n=18)	Mean Conc.	% Accuracy	% CV
LLOQ QC (0.20 pg/mL)	0.21	105.43	11.55
LQC (1.57 pg/mL)	1.55	98.82	9.82
MQC (10.00 pg/mL)	10.14	101.43	9.05
HQC (50.00 pg/mL)	50.62	101.24	5.99

4-4. Recovery

Extraction recovery was assessed across three QC levels, with formoterol recovery ranging from 58.42% to 64.85%. The %RSD remained under 15%. Results in Table 5 & 6 indicates the recovery statistics and global recovery of 62.09% for formoterol. Recovery was found precise, consistent and reproducible at all QC levels.

Table 5 Recovery

Sr.No.	Ext- Sample	PE-Sample	Ext- Sample	PE-Sample	Ext- Sample	PE-Sample
	LQC		MQC		HQC	
1	41,604	64,591	3,43,507	5,37,660	18,07,964	28,65,374
2	46,653	72,793	3,21,080	5,44,004	16,34,704	28,97,701
3	43,708	73,741	3,43,610	5,29,191	16,58,957	27,33,750
4	40,121	68,189	3,32,688	5,06,571	15,86,493	28,23,271
5	48,724	67,748	3,36,506	5,23,299	15,64,788	27,71,630
6	45,982	64,346	3,18,991	5,28,119	16,74,796	29,03,347
AVERAGE	44,465	68,568	3,32,730	5,28,141	16,54,617	28,32,512
STD DEV	3,251.35	3,976.64	10,707.57	12,900.07	86,079.02	69,127.51
% RSD	7.31	5.80	3.22	2.44	5.20	2.44
% Recovery	64.85		63.00		58.42	

Note: Read Ext-Sample as extracted sample and PE-Sample as post extracted sample

Table 6 Global Recovery

QC level	**Recovery**
LQC (n=6)	64.85
MQC (n=6)	63.00
HQC (n=6)	58.42
Mean	**62.09**
SD	**3.31**
% RSD	**5.33**

4-5. Matrix effect

Matrix factor was evaluated by comparing mean peak area ratio of QC sample in presence of matrix compared with mean peak area ratio of QC samples in absence of matrix. Post-extracted QC samples were prepared by extracting blank plasma from six different human plasma lots, followed by reconstitution with aqueous LQC and HQC samples. Post-extracted quality control samples and neat solution of quality control samples at LQC and HQC levels were analyzed on LCMS system. The IS normalized matrix factor at LQC and HQC levels were found 1.03 and 0.99 respectively, which is within acceptance criteria. Results of matrix effect were presented in Table 7.

Table 7 Matrix Factor

Formoterol	Response ratio in AQ- sample	Response ratio in PE- sample	Matrix factor	Formoterol	Response ratio in AQ- sample	Response ratio in PE- sample	Matrix factor
LQC	0.88	0.85	1.04	HQC	1.07	1.04	1.03
	0.72	0.77	0.94		1.05	1.02	1.03
	0.74	0.76	0.98		1.06	1.06	1.00
	0.75	0.74	1.01		1.05	1.08	0.98
	0.80	0.75	1.07		1.06	1.08	0.98
	0.88	0.76	1.15		0.87	0.94	0.92
Mean			**1.03**				**0.99**
SD			**0.08**				**0.04**
% RSD			**7.45**				**4.23**

Note: Read AQ-Sample as aqueous sample and PE-Sample as post extracted sample

4-6. Carry-over effect

Carryover was evaluated by injecting extracted samples in the sequence of extracted blank, extracted highest calibrator, extracted blank and extracted lowest calibrator. No carryover was present/observed at the retention time and MRM transition of the analyte in the extracted blank sample following the highest standard calibrator.

5. Conclusion

In this study, a sensitive, rapid, and less plasma volume LC-MS method was developed and validated for the quantification of the formoterol in human plasma. The method utilized a simple sample preparation and provided a short chromatographic runtime, enabling efficient high-throughput analysis. The assay demonstrated excellent analytical performance characteristics, including good linearity (0.2 pg/ml to 100 pg/ml, precision, accuracy, and selectivity. The method's low LLOQ of 0.2 pg/ml and minimal matrix effects make it a valuable tool for supporting clinical pharmacology and drug development applications where rapid data generation and minimization of sample volume are critical requirements.

Reference

1) https://www.chemspider.com/Chemical-Structure.2340731.html (Accessed Feb 13,2024)